

Genotype and phenotype of N-acetyltransferase 2 (NAT2) polymorphism in patients with contact allergy

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We investigated whether patients with contact allergy differed from non-contact-allergic, non-atopic controls with regard to genotype and phenotype of the polymorphic enzyme N-acetyltransferase 2 (NAT2). 55 contact-allergic patients recruited from the Information Network of Departments of Dermatology (IVDK) were compared to 85 controls from among local health care personnel. NAT2 activity was calculated from HPLC analysis of the ratio of the caffeine metabolites 5-acetylamino-6-formylamino-3-methyluracil (AFMU) and 1-methylxanthine (1MX) in the urine. NAT2 genotype was determined by polymerase chain reaction (PCR). A statistically significantly increased proportion of rapid acetylators was found in contact-allergic patients. This may have 2 possible implications: acetylation may enhance contact sensitization; or NAT2 status may be a genetic marker for contact sensitizability.

Key words: aryl amines; acetylator phenotype; acetylator genotype; allergic contact dermatitis; genetic susceptibility; molecular epidemiology; clinical epidemiology. © Munksgaard, 1998.

Accepted for publication 29 December 1997

It has been shown that allergic patients, atopics (1), contact-allergic patients (2), and patients with sulfonamide hypersensitivity (3) display a dominance of slow (or intermediate) N-acetyltransferase (NAT) activity. We wondered whether contact-allergic patients differed from controls (non-allergics) with regard to genotype and phenotype of the polymorphic enzyme NAT2. The individual phenotype of NAT2 is determined by caffeine metabolites, whereas NAT1 activity is measured by sulfonamide (sulfamethazine) acetylation. Here we report a statistically significantly increased proportion of rapid acetylators in contact-allergic patients.

Patients and Methods

From various departments of dermatology in Germany belonging to the IVDK (4), 55 patients sensitized to para-substituted aryl compounds (para-phenylenediamine (PPD), isopropyl-para-phenylenediamine, benzocaine, para-aminobenzoic acid

derivatives, p-aminophenol, para-toluylenediamine, sulfanilamide, Disperse Orange 3, para-aminoazobenzene, 4,4'-diaminodiphenylmethane) and/or sensitized to glyceryl-monothioglycolate (GMT) were identified by the central register of the IVDK, and invited to participate in the study with informed consent. 85 employees from among local health care personnel, without contact allergies and without signs of an atopic disease, served as controls. Patients and controls were asked to take 2 cups of coffee in the morning. The urine was collected 4 h later, and stored at a temperature of –20°C. Samples of venous blood were drawn. Within 24 h urine and blood were sent to Göttingen. NAT2 activity was calculated by the ratio of caffeine metabolites in the urine (5), namely 5-acetylamino-6-formylamino-3-methyluracil (AFMU) and 1-methylxanthine (1MX), which were analysed by HPLC. NAT2 genotype was determined by polymerase chain reaction (PCR/RFLP), as described by Cascorbi et al. (6, 7). The

study design was approved by the local ethics committee of the University of Göttingen.

Results

Based on the bimodal distribution of enzyme activity in our population, expressed as the ratio of the caffeine metabolites AFMU and 1MX, the cut-off point separating rapid and slow acetylators was placed at the quotient=1 and the logarithmic value=0, respectively (Fig. 2 and Fig. 1). In contact-allergic patients 25 (48%) were slow and 27 (52%) were rapid acetylators, whereas in the control group 65 (76,5%) were slow and 20 (23,5%) rapid acetylators (Fig. 1). In 3 patients, the material was not suitable for phenotypical determination. The difference between patients and controls was statistically significant (Fisher's exact test (2-tailed): $p < 0.001$). There was a good correlation between NAT2 phenotype and NAT2 genotype determined by PCR/RFLP (Fig. 2), with a concord-

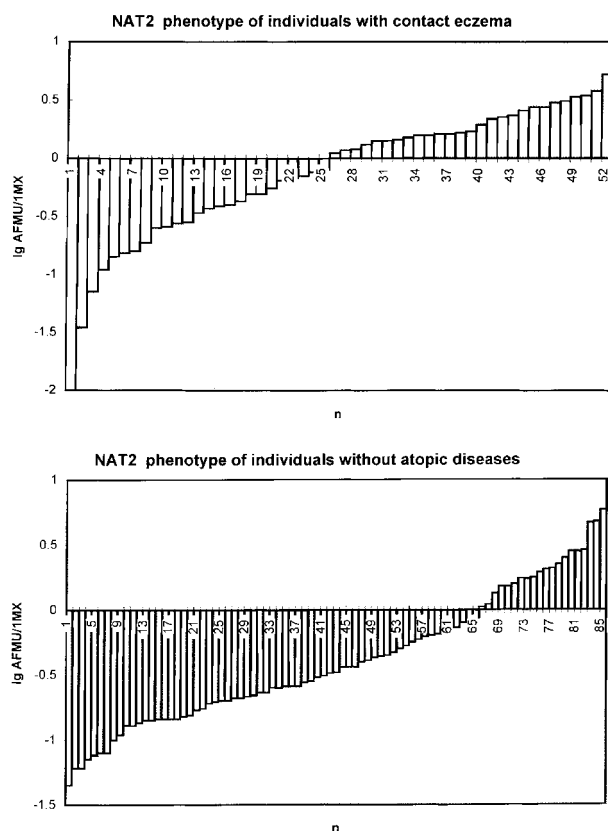
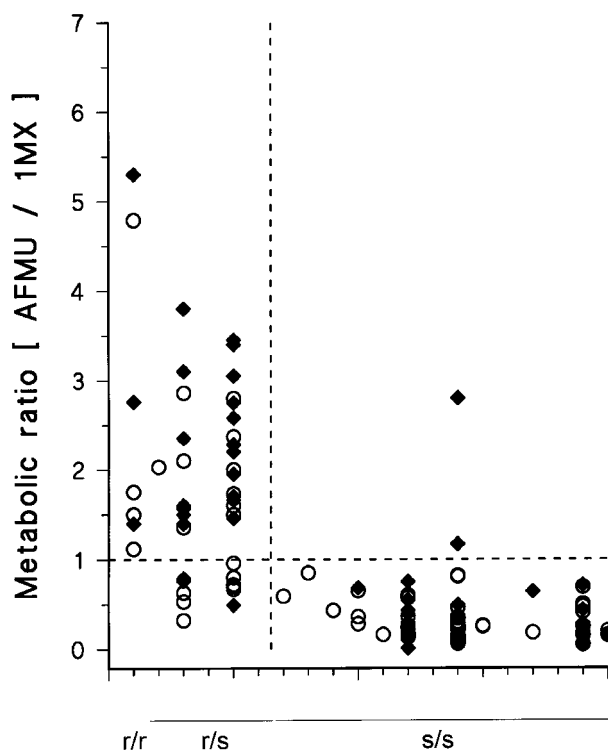


Fig. 1. Ratio of the caffeine metabolites AFMU and 1MX in 52 patients with contact allergy and 85 controls without allergy and without atopic diseases. The values were logarithmically transformed and the cut-off point separating slow (ratio<0) and rapid (ratio>0) acetylators was set at 0, according to the bimodal distribution of enzyme activity in the total study population. The difference between study and control groups was significant (Fisher's exact test (2-tailed): $p < 0.001$).



combination of alleles

Fig. 2. Relation between NAT2 genotype and phenotype: Ratios of AFMU/1MX metabolites related to the pattern of allele combinations in patients (black diamonds) and controls (open circles). r/r: homozygous rapid, r/s: heterozygous rapid, s/s: homozygous slow acetylators. Left of the vertical dotted line: genotypically rapid acetylators. Metabolic ratio>1: phenotypically rapid acetylators.

ance of >90%. In contact-allergic patients 51% were genotypically rapid acetylators, in the control group 31%.

Discussion

It is still not known why some people are prone to be sensitized to contact allergens. Polysensitization (sensitization to more than 1 allergen) may be a characteristic of a subgroup with increased susceptibility (8). A genetic background may play a role (9), but its influence is still poorly understood. Studies on genes of the HLA class II complex are inconclusive (10, 11). Individuals with increased susceptibility cannot yet be identified *before* being sensitized.

The pathogenetic steps of sensitization and elicitation in allergic contact dermatitis could be differentiated as 'pre-immunologic' and immunologic (e.g., antigen processing by dendritic cells). The former steps may comprise permeation and metabolism of a chemical from, e.g., a prohaptens to

a hapten (12, 13). This metabolism is catalyzed by enzymatic processes (14, 15). Some enzymes known (or suggested) to be involved in the metabolism of allergens are subject to genetic polymorphism (16). Kawakubo et al. (2) showed that patients with para-phenylenediamine allergy tended to have slow or 'intermediate' enzyme activity. As the controls (PPD-negative patients) were patients with eczema (and not non-allergics), these results are difficult to compare with our findings.

In our study, we were able to show that patients sensitized to para-substituted aryl compounds or GMT were characterized by a greater proportion of rapid acetylators compared to non-allergic controls. This difference was present in both genotype and phenotype polymorphism. The small discrepancy between phenotype and genotype determination in our study has already been noted by others, and may be explained by biological variability in phenotype expression of certain genotypes (7). The difference in the acetylator status between patients and controls may have 2 possible implications: acetylation of the above-mentioned compounds in the skin may enhance sensitization (functional interpretation); or the enzyme status of people may be 1 of perhaps several genetic markers of increased susceptibility to contact allergies, at least to certain compounds (genetic interpretation). Ongoing studies will focus on other genetic enzyme polymorphisms. Preliminary results of, e.g., glutathione S-transferase T1 genotype determinations in the same study and control groups led us assume that GST-T1-negative individuals were less frequent in contact-allergic patients (about 10% versus 20% in the control group), but these results are not yet sufficiently robust due to the small size of the study group. The investigation of several genetic polymorphisms may help elucidate the (pre-immunologic) pathogenesis and the genetics of contact allergies.

Acknowledgements

The study was supported by the P. G. Unna-Stiftung, Göttingen, and the GEPA, Göttingen, Germany.

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